PB85-144152

Heterotrophic Bacteria in Potable Water Progress in Method Options and Monitoring Benefits

(U.S.) Environmental Protection Agency
Cincinnati, OH

Jan 85

U.S. Department of Commerce
National Technical Information Service
NTIS

PB85-144152

EPA-600/D-84-296
January 1985

HETEROTROPHIC BACTERIA IN POTABLE WATER:

PROGRESS IN METHOD OPTIONS AND MONITORING BENEFITS

Donald J. Reasoner, Research Microbiologist
Edwin E. Geldreich, Chief
Microbiological Treatment Branch
Water Engineering Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency

WATER ENGINEERING RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA-600/D-84-296		PB85-144152

4. TITLE AND SUBTITLE	5. REPORT DATE
Heterotrophic Bacteria in Potable Water: Progress in Method Options and Monitoring Benefits	January 1985
	6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
Donald J. Reasoner and Edwin E. Geldreich	

9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Microbiological Treatment Br., Drinking Wtr. Res. Div. WERL, U.S. Environmental Protection Agency 26 West St. Clair St. Cincinnati, OH 45268	CBNC1A
	11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Water Engineering Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Cincinnati, OH 45268	14. SPONSORING AGENCY CODE EPA/600/14

15. SUPPLEMENTARY NOTES Project Officer Donald J. Reasoner, Research Microbiologist
Microbiological Trmt. Br., DWRD, WERL, USEPA
26 W. St. Clair St., Cincinnati, OH 45268

16. ABSTRACT

Measurements of heterotrophic bacteria in potable water can provide useful information to water plant operators and sanitary engineers. Bacterial density changes during treatment and quality deterioration during distribution can be monitored using heterotrophic plate count (HPC) methods. Consistent application of the HPC method selected will provide baseline data for assessing bacterial quality changes. Discussion will focus on optimizing the measurement of heterotrophic bacteria through choice of procedures, media, and incubation time and temperature.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
Release to Public	Unclassified	14
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

EPA Form 2220-1 (9-73)

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

HETEROTROPHIC BACTERIA IN POTABLE WATER: PROGRESS IN METHOD OPTIONS AND MONITORING BENEFITS

Donald J. Reasoner, Research Microbiologist
Microbiological Treatment Branch
U.S. Environmental Protection Agency
Cincinnati, Ohio 45268

Edwin E. Geldreich, Chief
Microbiological Treatment Branch
U.S. Environmental Protection Agency
Cincinnati, Ohio 45268

ABSTRACT

Measurements of heterotrophic bacteria in potable water can provide useful information to water plant operators and sanitary engineers. Bacterial density changes during treatment and quality deterioration during distribution can be monitored using heterotrophic plate count (HPC) methods. Consistent application of the HPC method selected will provide baseline data for assessing bacterial quality changes. Discussion will focus on optimizing the measurement of heterotrophic bacteria through choice of procedures, media, and incubation time and temperature.

INTRODUCTION

A means of measuring the general bacterial population in water has been included in Standard Methods for the Examination of Water and Wastewater (1) since the first edition in 1905. At that time, it was known as Standard Methods of Water Analysis. Early plate count procedures utilized nutrient gelatin plates with incubation at 20°C because gelatin liquifies at temperatures greater than 20°C. Only after the advent of agar as a gelling agent was the use of incubation temperatures greater than 20°C possible for the bacterial plate count. Thus, the second edition of Standard Methods (1912) introduced the 37°C, 24 h plate count using nutrient agar, but also allowed for a 20°C, 48 h plate count using nutrient gelatin. Inclusion of the 20°C plate count continued through the 12th Edition of Standard Methods (1965) but was dropped from the 13th through the 15th editions. The current 35°C standard plate count (SPC) procedure evolved from earlier methodology employing 37°C incubation for the "body temperature count" that was specified in Standard Methods from the 3rd edition, 1917 through the 8th edition, 1936, and the 9th edition of 1946 provided for 35-37°C incubation for 24 $\pm$ 2 h.

During the past few years, interest in measuring the bacterial density of potable water samples has resulted in some changes in the accepted procedure. Whereas in the past the bacterial plate count was referred to as the "total plate count" or the "standard plate count," the terminology in the forthcoming 16th edition of Standard Methods is the "heterotrophic plate count." Adoption of this name recognizes the fact that a bacterial plate count procedure does not provide a total bacterial count measurement and that, while stan-

dardized conditions are needed, there should be some latitude in the choice of the standardized conditions used so that the measurement is customized to the informational needs of the user.

Method 907, Heterotrophic Plate Count, of the 16th edition of Standard Methods provides the user with several choices for determining the bacterial density of water. The 16th edition of Standard Methods revives the 20°C incubation option and introduces the membrane filter and spread plate procedures for bacterial enumeration. It also recognizes the use of a low-nutrient medium for the heterotrophic plate count as well as changes in the length of incubation required for optimum enumeration at 20°C or 28°C. These changes in the bacterial plate count procedure have been made as a result of research that began in the mid-1970's and continues yet today.

The heterotrophic plate count (HPC) is a simple procedure whether done as a pour plate, a spread plate or a membrane filter (MF), and is a very useful tool. While not essential for assessing the safety of potable water, the HPC results provide information that supplements the total coliform results. The HPC can be used to indicate the general bacterial composition of the source water and the efficacy and efficiency of water treatment processes such as settling, coagulation, filtration and chlorination. Monitoring the HPC of the finished distribution water can provide information on the cleanliness of the distribution system, post-treatment bacterial aftergrowth and the effects of changing water temperature and chlorine residual on the bacterial population.

METHODS OPTIONS

Table 1 is a summary of the HPC methods options included in the 16th edition of Standard Methods. For the first time, Standard Methods offers the analyst a choice of three methods and two new media for the HPC procedure. The media formulations for the alternative HPC procedures are shown in Table 2.

Pour Plate Method (PPM). The pour plate method will continue as a HPC method for water samples even though it has some disadvantages that limit the recovery of bacteria. Because it uses melted agar which must be tempered to 44° to 46°C, the bacteria in the water sample are subjected to heat stress that may be severe enough to render the organisms non-viable. Additionally, since a nutritionally rich medium is used, physiologically stressed or injured bacteria may be unable to grow due to some form of metabolic inhibition. Both the heat stress and the rich growth medium contribute to reduced recovery of bacteria. Another draw back is that a maximum of only 1.0 ml of sample can be analyzed by this method.

The Spread Plate Method (SPM). Offered as an alternative HPC procedure, the spread plate method eliminates the heat stress of the tempered melted agar used in the pour plate method. The bacterial colonies grow on the surface of the agar rather than embedded in the agar as in the pour plate procedure, and this favors the development of the aerobic bacteria which may not develop well, or at all, when embedded in the agar. When an incubation period longer than 3 days

is used, better development of pigmentation in bacterial colonies generally occurs regardless of the medium used. Plates may be prepared ahead of time and stored for up to a week as long as the water loss per plate doesn't exceed 2 to 3 g. The maximum sample volume that can be examined is 1.0 ml, but generally 0.1 ml to 0.5 ml is best. With the richer growth media, spreaders can be a problem, but with R2A medium (2) this is generally not the case.

The spread plate method is not a new procedure. It has been around for many years and has been shown to provide higher plate counts than the pour plate procedure. In a comparison of results from 571 water samples collected from dead-end water mains during routine flushing, the average ratio of the 35°C pour plate result to the spread plate result was 0.24 (3). In other words, the mean spread plate result was about 4 times greater than the mean pour plate result. Additionally, the spread plate was shown to be more precise than the pour plate method. When the distribution of bacterial counts from the same sample was examined by both the spread plate and pour plate methods, it was found that nearly 90% of the pour plate counts were in the $\leq$ 500/ml category compared to only 49% of the spread plate counts (Table 3). Furthermore, only 6.3% of the pour plate counts were in the > 1000/ml compared to nearly 35% of the spread plate counts. This again illustrates that the pour plate method is very inefficient and may significantly underestimate the number of bacteria in a treated water sample. There are two effects that contribute to reduced counts by the pour plate method. First, the melted tempered agar causes irreparable injury to some of the bacteria. Second, growth of strongly aerobic bacteria is interferred with when the organisms are embedded in the agar rather than on the surface.

The Membrane Filter Method (MFM). This method is introduced for the first time for determination of the HPC (4, 5). The membrane filter technique is one of the water microbiologist's most versatile tools and most analysts are familiar with the membrane filter procedure as used for the total coliform determination. Use of the membrane filter method for the HPC allows the examination of large volumes of low turbidity waters and is particularly useful when the HPC is low, such as may occur with chlorinated effluent from a clearwell, or effluent from a reverse osmosis unit. With the m-HPC medium spreaders can be a problem; this is less frequently the case with R2A medium. Since m-HPC medium is not a sterile medium, contamination may occur and plates should be used within a day or two after preparation; m-HPC plates should be stored at refrigerator temperature until used. R2A medium is prepared as a sterile medium and does not have this drawback. The membrane filter procedure has the advantages of saving time, incubation space and materials as well.

Counting colonies on white membrane filters can be a problem because of small colony size for some bacteria and the lack of contrast between non-pigmented colonies and the white membrane. The problem can be alleviated somewhat by adjusting the light source to a low angle to create a shadowing effect, or by tilting the plate and refocusing the microscope. The latter manipulation requires con-

tinuous readjustment of the microscope, but it works well for counting the gridded membrane filters.

MONITORING CONSIDERATIONS AND BENEFITS

The information gained from bacteriological testing of water must be examined in conjunction with thorough knowledge of conditions at the source of supply, throughout the treatment process and in the distribution system. A single laboratory test, however favorable the result, does not justify the conclusion that everything is satisfactory. The value of bacteriological tests depends upon their frequent and regular application and examination of the results. Frequent examination using a simple bacteriological test such as the HPC analysis, in addition to the required total coliform test, will provide a bacteriological quality baseline that can be used as a reference. Changes in the bacteriological quality trend can then be determined and if the change is for the worse, remedial action can and should be taken. Improvement in bacteriological quality should also be noted and factors responsible should be identified, if possible, so that the conditions can be maintained.

Although we generally rely on the coliform test to indicate that contamination has occurred, the HPC can provide similar information. When there is a sudden rise in the HPC the analyst should alert the treatment plant operator and steps should be instituted to try to determine the cause of the increased HPC. If no change in quality of the water leaving the treatment plant is noted but the HPC shows an increasing trend at locations in the distribution system, then problems such as low chlorine residual, low pressure, back siphonage, or leaks in mains and service reservoirs may be suspected.

Figure 1. illustrates typical differences in HPC results obtained using media and methods alternatives in the 16th edition of Standard Methods for examining potable water samples. Plate count agar (SMA), regardless of method, gave the poorest results at 35°C of all the media used. Also, 35°C incubation, no matter what medium/method was used for the HPC yielded the lowest plate counts.

Figure 2. compares HPC results after 7 days of incubation for unchlorinated source water and chlorinated potable water samples. Colony counts were obtained using standard methods agar and R2A medium in the spread plate and membrane filter procedures and the m-HPC membrane filter procedure. This figure shows that 20°C incubation yielded the highest counts on all media for both types of samples. It also shows that 35°C results were very low compared to the 20°C results for the chlorinated water. In addition, the method used for the HPC of the chlorinated water at 35°C was more important than for the 20°C HPC. Thus, this data serves to underscore the fact that the choice of the HPC method/medium combination chosen should be determined experimentally by comparison testing and not by assuming that all the medium/methods combinations will yield equivalent results.

In order to use the HPC information in assessing overall treatment efficacy and distribution system conditions, the HPC baseline

data should be collected using the same medium/method combination throughout. Even when the HPC is very low, the membrane filter method provides a means of measuring the bacterial population by using sample volumes much larger than one milliliter. It is obvious from Figure 1 that a smaller subpopulation of heterotrophic bacteria is measured at 35°C than at 20° or 28°C; in fact only about $< 10\%$ in most cases. For baseline data, it is better to measure the largest population possible and to watch for any increasing trend as a sign that something has happened (or is happening) that allowed the HPC population to increase. Increasing or decreasing trends must be interpreted in light of seasonal changes in water quality, temperature and other factors. Only when adequate baseline data has been collected will the operator be able to clearly identify the seasonal factors that affect the bacteriological quality of his/her product. Knowledge of the changes that occur seasonally, or are associated with heavy precipitation events will enable the plant operator to predict or anticipate when the HPC will rise and to alter treatment sufficiently to minimize the bacteriological change.

Aside from seasonal changes, it may be possible to correlate increased HPC counts with the lengths of filter runs for plants practicing rapid sand filtration. In practice, the need to backwash a rapid sand filter bed is based on head loss, and the delay in obtaining the bacteriological results would not assist in the short term management of rapid sand filter operation. However, over the long term, knowledge about changes in the HPC related to rapid sand filter operation should be helpful to the plant operator or engineer in understanding and interpreting the bacteriological data. For treatment employing granular activated carbon (GAC) filtration, monitoring the HPC of the GAC filter bed effluent may be very beneficial. GAC filters develop very large bacterial populations ($>10^8$ cells/g wet wt. GAC), and changes in influent water quality characteristics such as temperature, pH and chlorine residual may cause increased numbers of bacteria to be shed into the GAC contractor effluent.

Since there is presently no mandatory requirement in the Primary Drinking Water Standards for the HPC measurement, the decision to monitor the HPC and the choice of methodology is strictly up to the water utility. It is hoped that an enlightened and progressive water utility management will choose to use the HPC as another informational tool to assist in optimal operation of the treatment process and production of a consistently high quality product for its customers.

REFERENCES

1. Standard Methods for the Examination of Water and Wastewater. APHA, AWWA, and WPCF. Washington, D.C. (15th ed., 1980).

2. Reasoner, D. J. and E. E. Geldreich. A New Medium for the Enumeration and Subculture of Bacteria from Potable Water. Appl. Environ. Microbiol. 49: (1985) in press.

3. Taylor, R. H., M. J. Allen and E. E. Geldreich. Standard Plate Count: A Comparison of Pour Plate and Spread Plate

Methods. Jour. AWWA, 75:35-37 (1983).

4. Taylor, R. H. and E. E. Geldreich. A New Membrane Filter Procedure for Bacterial Counts in Potable Water and Swimming Pool Samples. Jour. AWWA, 71:402-405 (1979).

5. Haas, C. N. et al. Analytical Note: Evaluation of the m-SPC Method as a Substitute for the Standard Plate Count in Water Microbiology. Jour. AWWA, 74:322 (1982).

6. Fiksdal, L. et al. Nonstandard Methods for Enumerating Bacteria in Drinking Water. Jour. AWWA 74:313-318 (1982).

Table 1. Summary of Heterotrophic Plate Count (HPC) Options in Standard Methods, 16th Ed., Section 907

Subsection	Medium	Incubation °C/Time	Sample Volume	Count Range
907 A				
Pour Plate Method (PPM)	TGE	35 ± 0.5°C/48 ± 3 h	1 ml maximum	30-300 CFU*
	PCA	35 ± 0.5°C/72 ± 4 ht		
	R2A	35 ± 0.5°C/72 ± 4 h; preferably 5-7 days 28° or 20°C/5-7 days		
907 B				
Spread Plate Method (SPM)	Same as 907 A	Same as 907 A	1 ml, but generally 0.1 to 0.5 ml is used	30-300 CFU
907 C				
Membrane Filter Method (MFM)	m-HPC	35 ± 0.5°C/48 ± 3 h	Variable, but ≥ 1 l may be filtered depending on sample turbidity	20-200 CFU
		35 ± 0.5°C/72 ± 4 ht		
	R2A	Same as 907 A		

*CFU = Colony Forming Units
† = Bottled Water

Table 2. HPC Media Formulations*, g/liter

Ingredient	Tryptone Glucose Yeast Extract Agar (TGE)	Plate Count Agar (PCA)	R2A Agar	m-HPC agar
Beef Extract	3.0	-	-	-
Tryptone	5.0	5.0	-	-
Glucose	1.0	1.0	0.5	-
Yeast Extract	-	2.5	0.5	-
Proteose Peptone #3 or polypeptone	-	-	0.5	-
Casamino Acids	-	-	0.5	-
Soluble Starch	-	-	0.5	-
K_2HPO_4	-	-	0.3	-
$MgSO_4 \cdot 7H_2O$	-	-	0.05	-
Sodium Pyruvate	-	-	0.3	-
Peptone	-	-	-	20.0
Gelatin	-	-	-	25.0
Glycerol	-	-	-	10.0
Agar	15.0	15.0	15.0	15.0
pH	6.8-7.0	7 ± 0.2	7.2	7.1
Dist. Water	1.0 L	1.0 L	1.0 L	1.0 L

*All media except m-HPC are sterilized by autoclaving at 121°C for 15 min. after heating to boiling to dissolve all ingredients
m-HPC agar is prepared by mixing all ingredients except glycerol, adjust pH with 1.0 N NaOH, heat to dissolve all glycerol and autoclave at 121°C for 5 min.

Table 3. Distribution of Bacterial Counts From the Same Sample Using Both the Pour Plate and Spread Plate Procedures

Procedure	Plate Count/mL		
	<500	500-1000	>500
Pour Plate			
No. of Samples	570	26	33
Percent	88.8	4.9	6.3
Spread Plate			
No. of Samples	259	85	185
Percent	49.0	16.1	34.9

From: Taylor, R. H., M. J. Allen, and E. E. Geldreich, Jour. Amer. Water Works Assoc. 75 (1): 35-37, 1983, with permission.

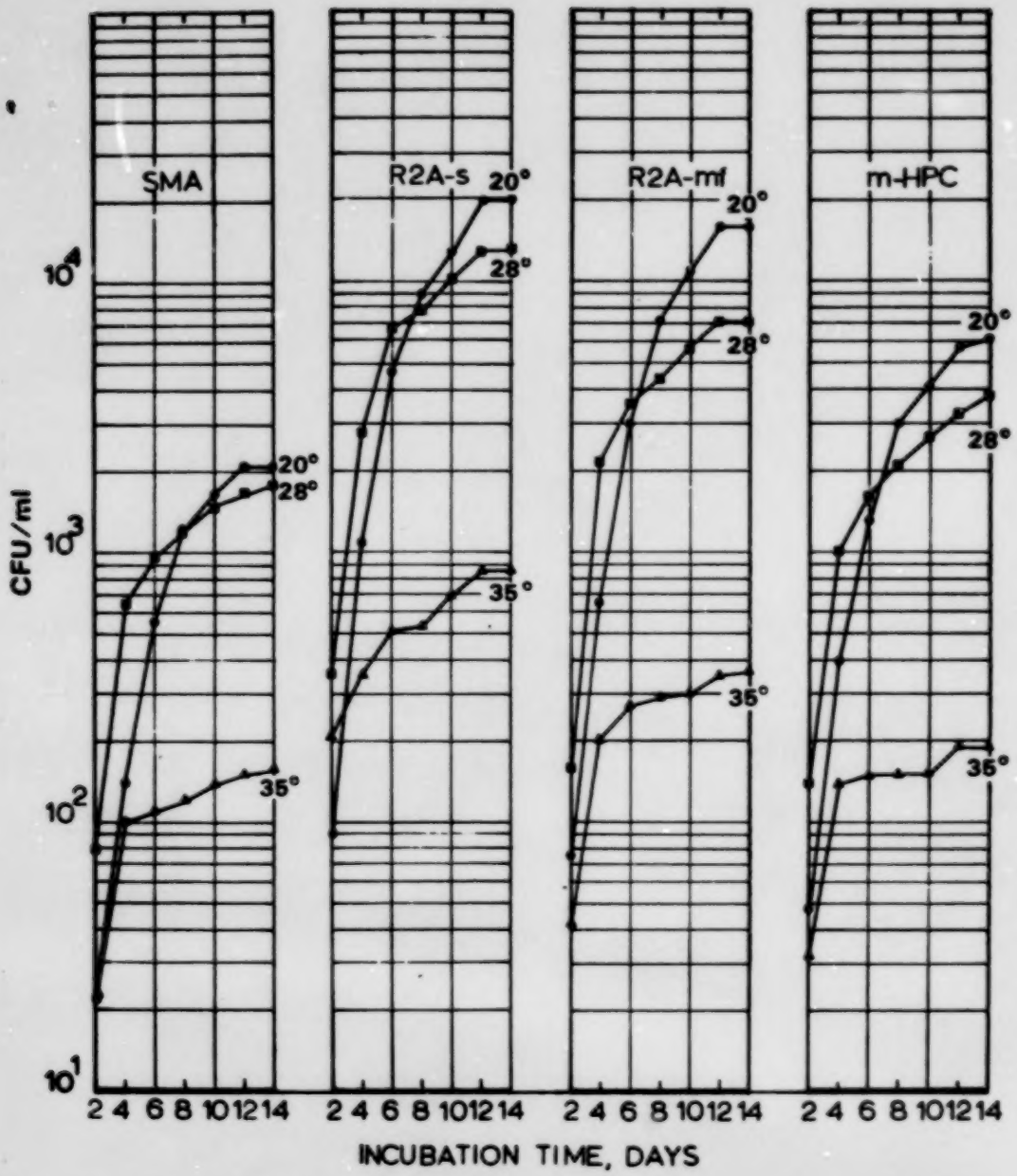

Figure 1. Comparison of 20^0, 28^0 and 35^0C Bacterial Counts (CFU/ml) of Treated Distribution Water. Colony Counts Obtained Using Media and Methods Options in Standard Methods (16th ed.) Section 907.

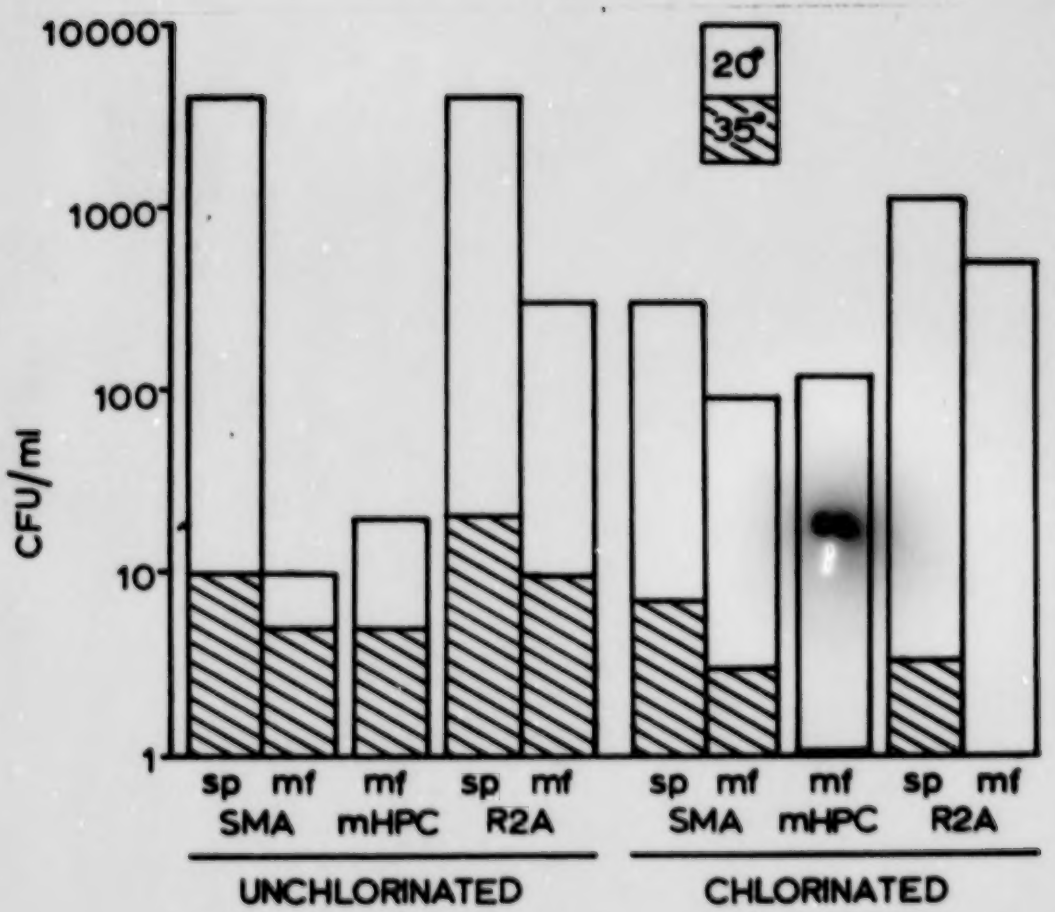

Figure 2. Comparison of 20° and 35° Bacterial Counts (CFU/ml) of Unchlorinated and Chlorinated Water. Counts Obtained Using the Spread Plate and Membrane Filter Methods, 7 Days Incubation and Three Different Media (after Fiksdal, et al., 6).